



Monthly Publication
ISSN: 0019- 462X

INDIAN DRUGS

Pandey V. P., Kudakasseril J., Cherian E. and Patani G

SCIENTIFIC & RESEARCH PUBLICATION FROM
INDIAN DRUG MANUFACTURERS' ASSOCIATION

Pharmaceutical Chemistry

Pharmacognosy,
Natural Products &
Phytochemistry

Biotechnology

Pharmaceutics

NDDS

Pharmaceutical Analysis

Pharmacology

Clinical Trials

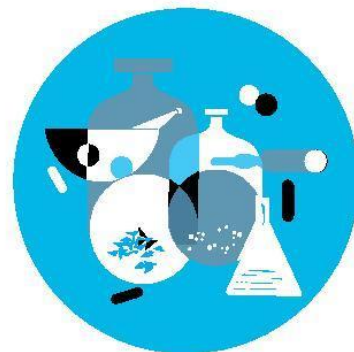
Genome Research

Stem cell Research

Vaccines & Sera

"COMPARISON OF TWO METHODS FOR IN VITRO
PROPAGATION OF RAUWOLFIA SERPENTINA FROM
NODAL EXPLANTS"

Year 2007, Volume 44 (7) p. 514-519



COMPARISON OF TWO METHODS FOR *IN VITRO* PROPAGATION OF *RAUWOLFIA SERPENTINA* FROM NODAL EXPLANTS

Pandey V. P., Kudakasseril J., Cherian E. and Patani G.*

Dr. Patani Scientific and Industrial Research, Inga Complex, PSIR building, Mahakali Road, Andheri (E), Mumbai 400093, India.

* Correspondent author's e-mail: psir79@gmail.com

Abstract

Two different methods of *in vitro* multiplication of *Rauwolfia serpentina* from nodal explants were compared viz. multiplication via callus morphogenesis and that via shoot proliferation from axillary buds. The second method was found to be far better. The optimum shoot proliferation occurred on Murashige and Skoog (MS) medium supplemented with 1 mg/L naphthalene acetic acid (NAA) and 2 mg/L of benzyl aminopurine (BAP). The best rooting of shoots occurred on MS medium containing 4% sucrose and 1 mg/L of NAA. Solid and liquid MS media were found to be similar in supporting shoot proliferation. The plants produced were successfully hardened and established in soil. An easy, reliable and reproducible protocol was developed for *in vitro* micropropagation of *Rauwolfia serpentina* from nodal explants.

Key words: *Rauwolfia serpentina*, Nodal explants, Multiple shoots, Micropropagation, Tissue culture, NAA and BAP

Introduction

Rauwolfia serpentina Benth (Sarpagandha) is an important medicinal plant belonging to family Apocynaceae, distributed in India, Pakistan, Burma and Thailand. The plant has significant economic importance due to the presence of indole alkaloids such as reserpine, serpentine ajmaline, rescinnamine and α -yohimbine, which are used in allopathic as well as ayurvedic systems for the treatment of hypertension, cardiovascular diseases and as a sedative tranquilizing reagent. Pharmaceutical companies largely depend upon materials procured from naturally occurring plants causing rapid depletion of this important medicinal herb. Hence, it has become imperative to establish a suitable protocol for micropropagation to generate sufficient plant material for the pharmaceutical industry without further endangering this species¹. Propagation of *Rauwolfia serpentina* through seeds is difficult because of poor germination². So an alternative is the use of tissue culture techniques for large scale multiplication of this plant.

Various attempts have been made for *in vitro* propagation of *Rauwolfia serpentina* using different explants. The propagation was achieved either through callus induction and its morphogenesis or through axillary bud proliferation, either using stem apices or nodal explants. Callus induction was reported by Perveen and Ilahi³ from stem pieces using either NAA, IBA or 2, 4-D. Tomar and Tiwari⁴ were able to get plants from callus of *Rauwolfia serpentina* cotyledons on a medium containing NAA and BAP. Akram and Ilahi⁵ obtained plants from calli induced from shoots of *Rauwolfia serpentina*. Sarker *et al.*⁶ were able to get shoot differentiation from callus of this plant using 0.5

mg/L BAP and 0.1 mg/L NAA. Sehrawat *et al.*⁷, reported the production of callus from leaf explant of *Rauwolfia serpentina* using 2 mg/L 2, 4-D and 0.2 mg/L BAP. Akram *et al.*⁸ obtained callus from roots of this plant using 1 mg/L IAA and 10 mg/L BAP. They succeeded in regenerating plants from the callus using 10% coconut milk and 5 mg/L biotin.

Multiple shoots were produced either from shoot apices or nodal explants. Jain *et al.*⁹ obtained multiple shoots from both types of explants on a combination of 0.5 mg/L IBA and 5.0 mg/L BAP. On the other hand Sehrawat *et al.*⁷ reported producing multiple shoots using a combination of BAP and NAA. Roja and Heble¹⁰ produced multiple shoots from axillary buds using a combination of 1 mg/L of BAP and 0.1 mg/L of NAA. Tiwari¹¹ was able to get multiple shoots from apical and nodal explants on MS medium supplemented with 3 or 5 mg/L of kinetin and 3 mg/L IAA. Roja *et al.*¹² obtained multiple shoots on a medium supplemented with 1 mg/L BAP and 0.1 mg/L NAA. Goel *et al.*¹³ reported on a cost-effective approach for *in vitro* mass propagation of *Rauwolfia serpentina* using shoot cultures. The purpose of our experiments was to develop an easy, reliable and reproducible protocol for large scale *in vitro* multiplication of *Rauwolfia serpentina*.

Materials and Methods

Nodal explants of *Rauwolfia serpentina* about 1.5 cm in length were collected from 2-3 year old plants. They were washed in running tap water and then washed again thoroughly with

distilled water containing 5-6 drops of Dettol and Tween 80. They were surface sterilized with 0.1% (W/V) mercuric chloride for 10 minutes followed by washing them five times with sterile distilled water. They were aseptically transferred to Murashige & Skoog's (MS)¹⁴ medium at pH 5.6 (adjusted prior to autoclaving), supplemented with growth regulators, 3% sucrose and 0.7% agar. The cultures were incubated at $25 \pm 2^\circ\text{C}$ with light intensity of 3000-Lux using white fluorescent lamps. A photoperiod of 16/8 light and dark cycle was maintained.

Two approaches were used for *In vitro* multiplication, either by callus morphogenesis or by multiple shoot formation. For callus induction nodal explants were cultured on MS medium containing different concentrations of BAP (0.5, 1, 1.5 and 2 mg/L) and 2, 4 dichlorophenoxy acetic acid (2, 4-D) (concentrations ranging from 0.1 to 3 mg/L). For shoot initiation nodal explants were cultured first on MS medium containing either kinetin, BAP or thidiazuron alone. In another experiment nodal explants were cultured on MS medium containing BAP (0.5, 1, 1.5 and 2 mg/L) and NAA (concentration ranging from 0.1 to 1 mg/L). In yet another experiment different auxins viz. Indole acetic acid (IAA), Indole butyric acid (IBA) and *para*-amino benzoic acid (PABA) (1 mg/L) were used in combination with either BAP (2 mg/L) or kinetin (2 mg/L). Callus was subcultured to MS medium containing 2, 4-D (concentration ranging from 0.5 to 5 mg/L) or NAA at similar concentrations. Callus was also subcultured to media containing thidiazuron (1, 2, 3, 4 and 5 mg/L) to induce shoot formation. Shoots produced from nodal explants were subcultured to liquid MS medium containing BAP and NAA (same concentrations as that of solid medium).

The shoots were induced to form roots on either Nitsch & Nitsch¹⁵ or MS medium supplemented with different concentrations of NAA (0.5, 1 and 2 mg/L). Two concentrations of sucrose were also used (3 and 4%). The rooted shoots were removed from the culture medium. After washing the roots with distilled water, they were transferred to plastic cups containing a mixture of steam sterilized garden soil, vermiculite and sand (1: 1: 1). The potted plants were grown under laboratory conditions of regulated humidity and temperature for two weeks. The plants were kept under shade for four weeks and then placed under full sunlight.

Results

In the first set of experiments nodal explants were placed on MS medium with combinations of 2,4-D and BAP for callus formation. MS medium with 3 mg/L

2,4-D and 2 mg/L BAP showed appreciable callus formation without shoot formation. Other combinations such as 0.1 and 1.0 mg/L of 2, 4 -D along with 1 and 2mg/L of BAP produced shoot formation with callus growth (Table 1). These calli when subcultured onto MS medium with different concentrations of 2,4-D showed more callus growth. Faster growth was obtained with 2, 4-D at 4 mg/L when compared to 2 and 3 mg/L. The calli were placed on MS media supplemented with 4mg/L thidiazuron for shoot formation. On thidiazuron containing media 4-5 shoots were produced from each piece of the callus after 15 days of inoculation, (Fig. 1). However, callus on 3mg/L IBA containing MS medium showed root formation (Fig. 2).

The MS media devoid of plant growth regulators and with BAP, kinetin or thidiazuron alone failed to support shoot formation from nodal explants. The first evidence of shoot induction was observed on MS media with the combination of 0.3 mg/L NAA and 2 mg/L BAP. The shoot buds first appeared after 20 days of inoculation. These shoot buds were subcultured onto MS medium supplemented with various concentrations of NAA and BAP for shoot multiplication. As is evident from Table 1 and Fig. 3 the maximum numbers of shoots were produced on medium containing 1 mg/L NAA and 2 mg/L BAP. Similar results were obtained on MS liquid media containing 1 mg/L NAA and 2 mg/L BAP. When the concentration of BAP was increased above 4 mg/L, the rate of shoot multiplication was reduced. Shoot multiplication on media containing kinetin or BAP along with different auxins other than NAA was poor (Table 1). The *in vitro* grown shoots placed on MS medium with 4% sucrose and 1 mg/L NAA, produced profuse rooting (Fig. 4 and Table 2). Root formation was slow on shoots inoculated on Nitsch and Nitsch medium containing NAA. The fastest rooting was obtained on MS medium containing 4% of sucrose compared to 3% in the control.

The survival rate of transplanted plant was 85%. The plantlets were sufficiently healthy with new growth (Fig. 5). They were subsequently transferred to larger pots and gradually acclimatized to outdoor conditions. The results indicate that the protocol reported here is reproducible and that it has great potential for use in large scale micropropagation of *Rauwolfia serpentina*.

Table 1. Effect of different combinations of growth regulators on shoot regeneration and multiplication from nodal explants of *Rauwolfia serpentina*.

Growth Regulators (mg/L)	*Regeneration Response (%)	**No. of Shoots/ Explant	**Shoot Length (cm)
BAP (0.5) + 2, 4 - D (0.3)	57	2.84 ± 0.27	3.8 ± 0.33
BAP (1.0) + 2, 4 - D (1.0)	65	^c 3.01 ± 0.74	4.1 ± 0.53
BAP (1.5) + 2, 4 - D (0.3)	50	4.24 ± 0.85	3.7 ± 0.41
BAP (2.0) + 2, 4 - D (1.0)	44	^c 1.93 ± 0.66	3.2 ± 0.63
BAP (0.5) + NAA (0.1)	52	3.83 ± 0.21	4.3 ± 0.15
BAP (1.0) + NAA (0.3)	57	5.17 ± 0.59	4.2 ± 0.19
BAP (1.0) + NAA (0.5)	65	8.44 ± 0.72	4.8 ± 0.20
BAP (1.5) + NAA (0.3)	59	11.09 ± 0.53	7.5 ± 0.31
BAP (2.0) + NAA (0.3)	71	14.61 ± 0.31	8.1 ± 0.28
BAP (2.0) + NAA (0.5)	75	16.04 ± 0.83	7.2 ± 0.59
BAP (2.0) + NAA (0.7)	65	16.93 ± 0.25	7.9 ± 0.21
BAP (2.0) + NAA (1.0)	73	19.81 ± 0.41	8.1 ± 0.28
BAP (2.0) + IAA (1.0)	43	2.03 ± 0.49	4.1 ± 0.44
BAP (2.0) + IBA (1.0)	35	2.23 ± 0.73	3.7 ± 0.58
BAP (2.0) + PABA (1.0)	22	1.09 ± 0.98	3.2 ± 0.72
Kinetin (2.0) + IAA (1.0)	53	0.95 ± 0.78	3.5 ± 0.84
Kinetin (2.0) + IBA (1.0)	18	1.52 ± 0.87	2.9 ± 0.37
Kinetin (2.0) + PABA (1.0)	10	1.83 ± 0.69	3.1 ± 0.55

* Average of 20 explants repeated thrice

** Mean ± SE (20 replicates repeated thrice)

c Callus growth

Table 2. *In vitro* rooting response of *R. serpentina* on MS media containing different concentrations of NAA along with two different concentrations of sucrose.

Media combination	*Rooting response (%)	**No. of roots/ explant
3 % sucrose and 0.5 mg/L NAA	64	32.03 ± 0.12
4 % sucrose and 0.5 mg/L NAA	85	47.19 ± 0.23
3 % sucrose and 1.0 mg/L NAA	73	59.32 ± 0.19
4 % sucrose and 1.0 mg/L NAA	81	84.41 ± 0.25
3 % sucrose and 2.0 mg/L NAA	53	23.13 ± 0.29
4 % sucrose and 2.0 mg/L NAA	59	32.56 ± 0.31

* Average of 20 explants repeated thrice

** Mean ± SE (20 replicates repeated thrice)



Fig 1. Multiple shoot formation in *R. serpentina* from callus on MS medium containing 4 mg/L thidiazuron



Fig 2. Rooting response of callus on MS medium containing 3 mg/L IBA



Fig 3. Multiple shoot formation in *R. serpentina* from nodal explant on MS medium containing 1 mg/L NAA and 2 mg/L BAP



Fig 4. Rooting response of *in vitro* shoot on MS medium containing 1 mg/L NAA and 4% sucrose

Discussion

Many studies have been done on *in vitro* micropropagation of *Rauwolfia serpentina* using different explants. When multiplication was achieved by callus morphogenesis explants used included immature cotyledons⁴, leaf⁷, stem⁵, and roots⁸. When multiplication was achieved by shoot proliferation, explants were either nodal segments¹⁰⁻¹³ or shoot apices⁷ or both¹¹. The most commonly used medium was that of Murashige and Skoog^{4-9, 11} while White's medium was used by some authors^{5, 10}. Ilahi and Akram¹⁶ used three kinds of media viz. MS, White's and Abou-Mandour's medium. Growth regulators used for callus induction included combinations of NAA and BAP^{4, 6}, NAA and kinetin⁵, 2, 4-D and BAP⁷ or auxins such as NAA, IBA or 2, 4-D alone³. We found that a combination of 2, 4-D and BAP was best in callus induction. For shoot formation combinations of growth regulators used included NAA and BAP^{6, 10, 12, 13}, NAA and kinetin¹¹ and IBA and BAP⁹. We found that a combination of BAP and NAA was the best. Even though many studies have been done by incorporating natural materials such as coconut milk, casein hydrolysate and yeast extract in the medium, we purposely avoided using these



Fig 5. Hardened plant of *R. serpentina*

materials for better reproducibility of our results.

Our studies have shown that multiplication via shoot culture is much better than via callus morphogenesis. BAP (2 mg/L) in combination with NAA (1 mg/L) gave the best results in shoot proliferation from nodal explants. This differs significantly from the most popular concentration used viz. 0.1 mg/L NAA and 1 mg/L BAP^{6, 10, 12, 13}. Our studies have also shown that thidiazuron (4 mg/L) gave the best shoot proliferation from calli. MS medium supplemented with 4% sucrose was found to be better than the normal 3% to promote fast root formation from shoots. Solid medium was found to be similar to liquid medium in promoting shoot proliferation. Root formation from shoots was optimal with NAA at 1 mg/L in our study. To conclude, we have developed an easy, reliable and reproducible protocol for rapid *in vitro* propagation of *Rauwolfia serpentina*, which differs significantly from other studies in the kind and the concentrations of growth regulators used.

References

1. Jeyakumar M. and Jayabalan N.: *In vitro* plant regeneration from cotyledonary nodes of *Psoralea corylifolia* L., **Plant Tissue Cult.** 2002, (12) 2; 125-129.
2. Mitra G. C.: Studies on the formation of viable and non-viable seeds in *Rauwolfia serpentina* Benth, **Indian J. Exp. Bio.** 1976, 14; 54-56.
3. Perveen R. and Ilahi I.: Callus formation in *Rauwolfia serpentina*, **Pakistan J. Bot.**, 1978, 10(2), 141-148.
4. Tomar R. and Tiwari S.: *In vitro* morphogenesis of *Rauwolfia serpentina* plants through lateral bud culture, **Plant Cell Biotech. and Mol. Biol.**, 2006, 7(1/2), 53-58.
5. Akram M. and Ilahi I.: *In vitro* propagation of *Rauwolfia serpentina* through stem tissue, **Pakistan J. Sci. and Ind. Res.**, 1985, 28(6), 412-416.
6. Sarker K.P. Islam A., Islam R. Hogue A. and Joarder O.I.: *In vitro* propagation of *Rauwolfia serpentina* through tissue culture, **Planta Med.**, 1996, 62(4), 358-359.
7. Sehrawat A.R. Uppal S. and Punia A.: *In vitro* culture and multiplication of *Rauwolfia serpentina* – A threatened medicinal plant, **Crop Res.**, 2001, 22(1), 68-71.
8. Akram M. Nazir R. and Afridi K.: Shoot regeneration from *Rauwolfia* root callus, **Hamdard Medicus**, 1993, 36(3), 34-40.
9. Jain A.K. Dongare N. and Dubey P.K.: *In vitro* plantlet regeneration in *Rauwolfia serpentina*, **Biotech.-An Indian J.**, 2007, 1(1).
10. Roja G. and Heble M.R.: Indole alkaloids in clonal propagules of *Rauwolfia serpentina*, **Plant Cell Tissue and Organ Culture**, 1996, 44(2), 111-115.
11. Tiwari S.K.: Micropropagation of economically important medicinal plant *Rauwolfia serpentina* through tissue culture from excised apical nodal explant, **J. Trop. Forestry**, 1995, 11(2), 121-124.
12. Roja G. Benjamin B.D. Heble M.R. Patankar A.V. and Sipahimalani A.T.: The effect of plant growth regulators and nutrient conditions on growth and alkaloid production in multiple shoot cultures of *Rauwolfia serpentina*, **Phytotherapy Res.**, 1990, 4(2), 49-52.
13. Goel M.K. Kukreja A.K. and Khanuja P.S.K.: Cost-effective approaches for *In vitro* mass propagation of *Rauwolfia serpentina*, **Asian J. Pl. Sci.**, 2007, 1-5.
14. Murashige T. and Skoog F.: A revised medium for rapid growth and bioassay with tobacco tissue culture, **Physiol. Plant**, 1962, 15, 473-497.
15. Nitsch J.P. and Nitsch C.: Haploid plants from pollen grains, **Science**, 1969, 163, 85-87.
16. Ilahi I. And Akram M.: *In vitro* propagation of *Rauwolfia serpentina* plants through lateral bud culture, **Pakistan J. Agri. Res.**, 1987, 8(2), 204-210.